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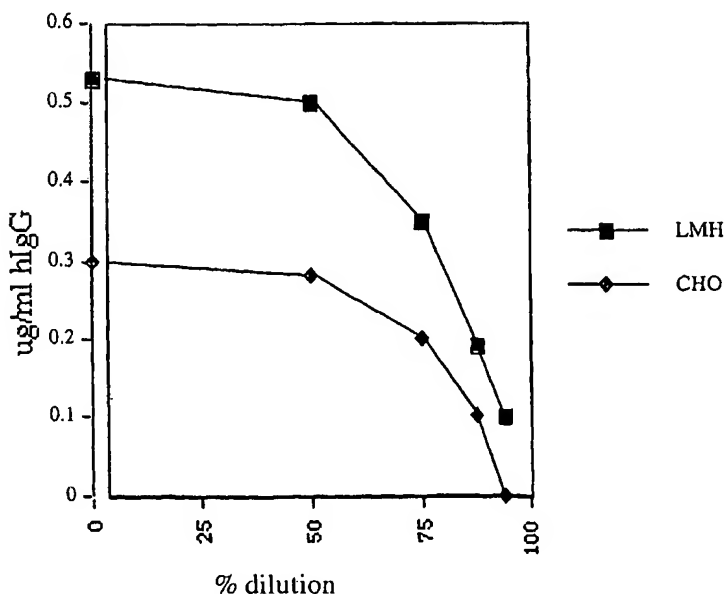
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[Continued on next page]

(54) Title: EXPRESSION OF MODIFIED ANTIBODIES IN AVIAN CELLS



(57) Abstract: The present invention relates to construct and methods which allow the expression of immunoglobulins or functional fragments thereof which have been altered so that they are humanised. The expression of the immunoglobulins or fragments thereof takes place in avian cells, and the constructs used have been altered such that the expression levels in avian cells are higher than what would have been expected by simply using a humanised construct. The alterations are based on changing codons so that each amino acid of the codon that is used is the one which is most often found in avians.

Concentrations of human IgG in culture medium from cells transiently transfected with 4ug p7.2

Concentration of chimaeric R24 minibody was determined by human IgG1 ELISA.

For % dilution, 0 = undiluted medium



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

1 Expression of modified antibodies in avian cells

2

3 The present invention relates to the expression of  
4 immunoglobulins, or more specifically, antibodies which  
5 have been altered so that the antibody is 'humanised', in  
6 avian cells. The antibody expression may occur either in  
7 vivo or in vitro. In the following document, the terms  
8 immunoglobulin and antibody are used interchangeably.

9

10 Antibodies are proteins of the immunoglobulin class which  
11 are produced on exposure to an antigen. The antibody  
12 produced recognises that antigen, binding selectively to  
13 it. There are five classes of immunoglobulin and the  
14 following text relates primarily to antibodies of the  
15 class IgG, although the other classes: IgA, IgD, IgY, IgE  
16 and IgM are also included. An antibody molecule is made  
17 up of two identical heavy chains linked by disulphide  
18 bonds and two identical light chains. The biological  
19 effector functions of an antibody molecule derive from  
20 the properties of a constant region, which is identical  
21 for antibodies of all specificities within a particular  
22 class. It is the variable region that contains the  
23 site/s which allows binding to a particular epitope and

1 there is a variable domain at the end of each of the  
2 heavy and light chains. These variable domains are  
3 followed by a number of constant domains. Thus, the  
4 binding of an antibody to an antigen occurs through  
5 interactions of the variable domains of each pair of  
6 heavy and light chains. Specifically, binding occurs in  
7 the areas of the variable regions where there is most  
8 variability. These regions are known as hyper variable  
9 regions or complementarity determining regions (CDRs).

10

11 Generally, with the exception of vaccinations, antibodies  
12 are generated from a non-human source such as a mouse.  
13 When used in human therapeutic applications, such  
14 antibodies are usually recognised as foreign by the  
15 immune system. This results in human anti-mouse  
16 antibodies being produced, which may reduce the  
17 therapeutic effect of the initial antibody or produce  
18 undesirable side effects. Techniques have been developed  
19 which allow the base of murine antibodies plus those of  
20 other species to be manipulated in a way that the  
21 original antigen specificity is retained, but all the  
22 non-essential parts of the immunoglobulin sequence are  
23 replaced with the equivalent human derived sequence.  
24 This is known as 'humanising' an antibody. By using  
25 humanised antibodies, immunogenic responses are largely  
26 or highly avoided and effector functions improved.

27

28 However, even when an immunoglobulin sequence is  
29 humanised there are still problems with the glycosylation  
30 pattern of immunoglobulins for use in humans which have  
31 not been produced in humans. IgG contains a conserved N-  
32 glycosylation site located within the CH2 domain of each  
33 heavy chain. The glycosylation pattern of

1 immunoglobulins is highly heterogeneous and has been  
2 shown to have a significant effect on the biological,  
3 pharmacological and physiochemical properties of the  
4 immunoglobulin and include stability of the antibody and  
5 half-life, tolerance in patient treatment and  
6 interactions with complement components and other Ig  
7 receptors. Alterations in the glycosylation pattern of  
8 IgG has been linked to symptoms of rheumatoid arthritis.  
9 The oligosaccharides are produced in the Golgi apparatus  
10 of the cellular interior and is regulated largely by the  
11 glycosyltransferases present in this organelle.  
12 Currently, antibodies for therapeutic applications are  
13 being produced in a variety of cell lines and  
14 transgenically, but many of these systems are not  
15 particularly suitable in terms of glycosylation. The  
16 same antibody produced in different cell lines and  
17 animals may therefore be afforded different  
18 characteristics which may result in differing functions  
19 and pharmacokinetics. The expression of  
20 glycosyltransferases differs with different cell types,  
21 with the result that the glycosylation pattern of the  
22 protein produced differs from that produced by other cell  
23 types. In particular, it has been noted that non-human  
24 mammalian cell lines, for example hamster cell lines,  
25 show markedly different glycosylation patterns to humans  
26 and therefore are likely to cause problems with  
27 immunogenicity. It is known that normal Chinese Hamster  
28 Ovary (CHO) Cells, which are the standard used in the  
29 industry for the manufacture of recombinant proteins, do  
30 not express the enzyme N-acetylglucosaminyltransferase-  
31 III (GlcNAcT-III), which has the role of synthesising  
32 carbohydrates which contain GlcNAc (Campbell & Stanley,  
33 1984). Since GlcNAc is expressed in human immunoglobulin

1 G, it is known that human proteins expressed in CHO cells  
2 are not glycosylated in this manner. It is also  
3 interesting to note that chicken IgG's also possess this  
4 oligosaccharide. It has been found by experts in the  
5 area, such as that reported by Raju et al. 2002, that the  
6 glycosylation patterns of avian cells are far more  
7 similar than non-human mammalian glycosylation patterns  
8 to human glycosylation patterns. It therefore can be  
9 seen that it would be highly advantageous to be able to  
10 utilise chicken cells as a mode of production for  
11 humanised recombinant proteins, compared to production of  
12 such proteins in the standard mammalian cells.

13  
14 US Patent No US5225539 entitled "Recombinant Altered  
15 Antibodies and Method of Making Altered Antibodies"  
16 describes a method of replacing the complementarity  
17 determining regions of the heavy or light chain variable  
18 domains of the receiving antibody with the corresponding  
19 complementarity determining regions of a different  
20 antibody with a different specificity. This method,  
21 known as 'CDR grafting' is not the only method to produce  
22 humanised antibodies, but is the most well known to those  
23 knowledgeable in the field. The present invention  
24 relates to antibodies which have been manipulated in any  
25 manner which has the result of producing an antibody  
26 which is more human-like in sequence than the wild-type  
27 sequence.

28  
29 However, although Patent No US5225539 describes a general  
30 method which allows altered antibodies to be produced,  
31 the methods described are directed towards production of  
32 the altered antibodies in mammalian cells and does not  
33 envisage the problems and advantages that arise when

1 producing antibodies in avian cells using this  
2 technology.

3

4 While the methodology described in Winters' Patent  
5 US5225539 is extremely useful for the production of CDR  
6 grafted antibodies in mammalian cells, it can be seen  
7 that it would also be useful to be able to easily produce  
8 such CDR grafted and humanised antibodies in avian cells.  
9 The production of such antibodies in avian cells would  
10 have a significant advantage over the production in  
11 mammalian cells if they are to be used as a human  
12 therapeutic. This is because the glycosylation pattern  
13 of human antibodies is more similar to avian antibodies  
14 than the glycosylation of mammalian antibodies. As  
15 previously mentioned, it is known that the glycosylation  
16 pattern can have a significant effect on the bioactivity,  
17 immunogenicity and therefore tolerance to the treatment  
18 and also the pharmacokinetics of the antibody itself, and  
19 therefore it would be extremely useful to produce  
20 antibodies for human use of which the glycosylation  
21 pattern is close to that of human antibodies.

22

23 It is also known that the yield of proteins produced in  
24 non-avian transgenic animals, can be limited. It is  
25 common practice to have to alter the culture conditions  
26 or use expression vectors in order to obtain commercially  
27 viable expression levels. The inventors have shown that  
28 the expression of such proteins, especially  
29 immunoglobulins and fragments thereof, in avian cells is  
30 in general, higher than that observed in non-avian  
31 animals. It can therefore be seen that it would be useful  
32 and more time and cost-effective to produce antibodies  
33 for human therapeutic use in avian cells. Figure 1 shows

1 the result of a Western blot to compare the expression  
2 levels of the R24 protein in Chicken Hepatocellular (LMH)  
3 cells, as compared to expression in Chinese Hamster Ovary  
4 (CHO) cells. Figure 2 illustrates a comparison between  
5 R24 protein produced in LMH and CHO cells, analysed by  
6 human IgG1 ELISA. It can be seen that there is a  
7 significantly higher level of protein produced in the LMH  
8 cells as compared to the CHO cells. The inventors  
9 believe that this is due to certain differences between  
10 the translational machinery of the cell types in that the  
11 LMH cells are more efficient in post-translationally  
12 modifying the protein, than mammalian cells. This  
13 belief is emphasised when the RNA message produced by  
14 both cell types is analysed by PCR gel and the levels  
15 produced by both types are similar.

16

17 One of the major problems of producing modified  
18 antibodies in avian cells is that the codon usage in  
19 avians differs from that in humans. Therefore, the  
20 methodology described in US Patent No 5225539 is not  
21 sufficient to allow the making of altered antibodies in  
22 avian cells efficiently.

23

24 There is also the problem of actually producing that  
25 which is coded for in a genetic construct in avian cells.  
26 US Patent No US4816397 describes methods of producing  
27 multi-chain polypeptides or proteins generally, however  
28 again the methods are discussed mainly with regard to  
29 mammalian cells and do not address the problems which  
30 occur when producing multi-chain polypeptides, such as  
31 antibodies, in avian cells. It should be noted that  
32 there are a number of Patents and Patent Applications  
33 which address the problem of producing basic single chain



1 proteins in avian cells, however in these cases they do  
2 not refer to the production of complex multi-chain  
3 polypeptides, such as antibodies, which pose their own  
4 problems.

5

6 It can therefore be seen that it would be beneficial to  
7 provide a method of producing and expressing modified  
8 antibodies which have similar glycosylation patterns to  
9 those naturally produced in humans or more similar than  
10 the glycosylation patterns obtained from antibodies from  
11 culture in mammalian cells.

12

13 It can also be seen that it would be beneficial to  
14 provide a method of producing and expressing modified  
15 antibodies in avian cells, as specifically producing and  
16 expressing humanised antibodies in avian cells, so that  
17 they can be used as human therapeutics.

18

19 It would also be extremely useful if the expression of  
20 the modified antibodies could be specifically in the egg  
21 of a genetically modified avian, so that the antibody can  
22 easily be collected and purified.

23

24 It is therefore a first object of the present invention  
25 to provide antibodies which have a glycosylation pattern  
26 which is more similar to naturally occurring human  
27 glycosylation patterns than those antibodies produced in  
28 mammalian cells.

29

30 It is a further object of the present invention to  
31 provide a method of expressing such humanised antibodies  
32 in avian cells *in vitro* and *in vivo*.

33

1 It is a further object of the present invention to  
2 provide a method of expressing humanised antibodies in  
3 avian cells which are known to produce higher yields than  
4 observed in non-avian cell systems.

5

6 It is a further object of the present invention to  
7 provide a method of expressing humanised antibodies in  
8 avian cells *in vitro* and *in vivo*.

9

10 It is a yet further object of the present invention to  
11 provide antibodies for therapeutic use, which have  
12 glycosylation patterns which are more similar to those  
13 naturally occurring in humans, than the glycosylation  
14 patterns observed in antibodies produced in mammalian  
15 cells.

16

17 A still further object of the present invention is to  
18 provide a construct which can be delivered into avian  
19 cells, which will allow the production of antibodies or  
20 humanised antibodies.

21

22 A final object of the present invention is to provide a  
23 method of expressing humanised antibodies in avian cells,  
24 so that they are specifically produced in the egg white  
25 or egg yolk of an avian.

26

27 According to a first aspect of the present invention,  
28 there is provided a DNA construct which when transfected  
29 into an avian cell will allow the production of an  
30 antibody molecule or functional fragment of said  
31 molecule, and which comprises at least one sequence which  
32 comprises the variable domain of an immunoglobulin heavy  
33 chain and at least one sequence which comprises the

1 variable domain of an immunoglobulin light chain, and  
2 wherein the DNA construct is based on a non-avian  
3 sequence and one or more of the codons in the DNA  
4 construct have been altered such that for the amino acid  
5 being encoded, the codon used is that which most  
6 frequently appears in avians.

7

8 Preferably, the construct also contains an avian signal  
9 peptide sequence.

10

11 Preferably the construct is cloned into a viral vector  
12 such as a lentivirus vector

13

14 Most preferably, the avian signal peptide sequence is a  
15 signal peptide sequence from an egg white protein such as  
16 lysozyme, ovalbumin, ovatransferrin or ovomucoid.

17

18 Most preferably, the construct also includes  
19 immunoglobulin constant regions for dimerisation and  
20 recruitment of effector functions.

21

22 Most preferably, the immunoglobulin constant regions are  
23 CH2 and CH3 of any IgG class.

24

25 Still more preferably, the immunoglobulin constant  
26 regions are human constant regions in order to provide a  
27 humanised antibody.

28

29 Preferably, the construct may be transfected into an  
30 avian cell using lipofection.

31

32 Alternatively, the construct is transfected into an avian  
33 cell using electroporation.

1

2 A further option is that the construct may be directly  
3 injected into the nucleus of an avian, into the germinal  
4 disc of an oocyte.

5

6 Preferably, codon usage in the construct is maximised for  
7 those codons most frequently appearing in avians. That  
8 is, each codon is altered so that it still codes for the  
9 same amino acid, but uses the codon most often found to  
10 code for that amino acid in avians.

11

12 According to a second aspect of the present invention,  
13 there is provided an avian cell, containing the construct  
14 of the first aspect, which expresses an immunoglobulin  
15 molecule or functional fragment of said molecule.

16

17 Preferably the expressed immunoglobulin molecule or  
18 fragment thereof shows an avian glycosylation pattern.

19

20 Preferably the immunoglobulin or fragment thereof is  
21 expressed at a higher expression level than a standard  
22 human construct or humanised construct.

23

24 According to a third aspect of the present invention,  
25 there is provided a method for producing avian cells  
26 capable of expressing an immunoglobulin molecule or  
27 functional fragment of said molecule, comprising  
28 transfecting an avian cell with the DNA construct of the  
29 first aspect.

30

31 Preferably the avian cell is a chicken cell, but may also  
32 be duck, turkey, quail, or ostrich.

33

1 According to a fourth aspect of the present invention  
2 there is provided an immunoglobulin or functional  
3 fragment thereof produced using the method of the third  
4 aspect.

5  
6 According to a fifth aspect of the present invention  
7 there is provided a transgenic avian, which expresses the  
8 construct of the first aspect.

9  
10 Preferably the antibody molecule, or functional fragment  
11 of said molecule, that is coded for by the construct is  
12 expressed in an egg of the transgenic avian.

13  
14 Most preferably the construct is expressed in the egg  
15 white.

16  
17 Alternatively the construct is expressed in the egg yolk.

18  
19 Preferably the immunoglobulin shows an avian  
20 glycosylation pattern.

21  
22 The present invention will now be illustrated, by way of  
23 example only, with reference to the following Figures in  
24 which:

25  
26 Figure 1 shows a Western blot showing the differences in  
27 protein expression between chicken and mammalian cells;  
28 and

29  
30 Figure 2. is a graph illustrating the differences between  
31 protein expression levels in chicken and mammalian cells,  
32 as analysed by ELISA; and

33

1 Figure 3 is a table giving the frequency of codon usage  
2 in chickens.

3

4 And with reference to the following sequences in which;

5

6 SEQUENCE ID 1 is the sequence of human IgG Fc used for  
7 construction of chimeric and humanised minibodies; and

8

9 SEQUENCE ID 2 is the sequence of the chickenised IgG Fc  
10 DNA sequence.

11

12 SEQUENCE ID 3 is the nucleotide alignment of the  
13 original and chickenised human IgG Fc.

14

15 SEQUENCE ID 4 is the amino acid alignment of original and  
16 chickenised human IgG Fc.

17

18 SEQUENCE ID 5 shows the chickenised R24 nucleotide  
19 sequence.

20

21 SEQUENCE ID 6 shows the complete chickenised nucleotide  
22 sequence of the R24 chimeric minibody

23

#### 24 Generating a Construct

25

26 In this example a construct is produced which allows a  
27 humanised murine R24 antibody to be produced in chickens.

28

29 The DNA encoding the single chain variable fragment is  
30 designed in *silico* so that it can then be directly  
31 synthesised using standard methods.

32

33 The starting sequences are human Vh and Vl sequences

1 which may be obtained from human IgM antibody. Vh and Vl  
2 complementarity determining regions (CDRs) of another  
3 immunoglobulin (which in this case is the murine R24  
4 immunoglobulin) are identified by standard methods (e.g.  
5 see Antibodies-Structure and Sequence at  
6 <http://www.bioinf.org.uk/abs>) and the R24 CDRs are  
7 swapped directly into the human immunoglobulin framework.

8  
9 The 3' end of the Vh DNA sequence is linked to the 5' end  
10 of the Vl DNA sequence by a (Gly<sub>4</sub>Ser)<sub>3</sub> peptide linker, as  
11 seen in SEQUENCE ID 1. Included at the 3' end of the Vl  
12 sequence is a sequence encoding a Bam HI restriction  
13 site. This gives the humanised R24 sequence 1. An IgG1  
14 leader sequence is linked to the 5' end of Vh with the  
15 inclusion of an Eco RI restriction site.

16  
17 To provide the constant region of the immunoglobulin 2,  
18 human IgG1 CH2/CH3 (Fc) DNA is then cloned by RT-PCR from  
19 RNA. The primers incorporate Bam HI and Sal I  
20 restriction sites and can be seen in SEQUENCE ID 2. The  
21 amplified DNA fragment is cloned directly following PCR  
22 using the PCR cloning vector pGEM-T (Promega). E coli  
23 DH5α cells are transformed with the ligated plasmid,  
24 plated out on amp selection media and colonies screened  
25 the following day by PCR with M13 vector primers.  
26 Positive clones with the appropriately sized insert can  
27 then be selected and plasmid DNA can be prepared and  
28 inserts sequenced to confirm the presence of  
29 immunoglobulin constant region DNA. The insert from one  
30 positive DNA clone is removed by Bam HI/Sal I digestion  
31 and ligated into pGEM 3Z (Promega), digested with the  
32 same restriction enzymes. After transformation and  
33 overnight growth on amp media, colonies are screened by

1 PCR with M13 vector primers and plasmid prepared from one  
2 positive clone.

3

4 One  $\mu$ g of the R24 gene synthesis product 1 is digested  
5 with *EcoRI/BamHI* and ligated into plasmid hFc digested  
6 with the same enzymes. After transformation and overnight  
7 growth on amp media, colonies are screened by PCR with  
8 M13 vector primers and plasmids prepared from clones with  
9 the appropriately sized insert.

10

11 The entire insert DNA is then removed from pGEM3Z by  
12 digestion with the restriction enzymes *EcoRI/SalI* and  
13 ligated into the mammalian expression vector pCIneo  
14 (Promega) digested with the same enzymes. After  
15 transformation and overnight growth on amp media,  
16 colonies are screened by restriction digestion  
17 (*EcoRI/SalI*) of plasmid preparations. Plasmids may be  
18 sequenced to confirm the presence of the minibody genes.

19

20 The insert can then be used to form a construct for  
21 insertion into an avian cell. The insert, comprising the  
22 Vh/Vl CDRs transplanted into a human immunoglobulin  
23 framework 1 along with immunoglobulin constant domains 2  
24 is removed from the pCIneo vector by *BglII/SfiI*  
25 digestion. The fragment that is gained by this digestion  
26 consists of;

27

28 a promoter/ enhancer 6,

29 an intron 4,

30 the minibody which comprises the R24 variable regions 1  
31 and the CH2 and CH3 constant regions 2, and

32 a poly A tail 3

33



1 In order for the construct to be suitable for expression  
2 in an avian cell, the immunoglobulin leader sequence is  
3 exchanged for an avian specific sequence such as the  
4 lysozyme signal peptide sequence 5. Also, both the R24  
5 variable section coding sequence 1 and the CH2/CH3  
6 constant region coding sequence 2 are chickenised.

7

8 'Chickenising' a Construct

9

10 Chickenising is defined as the alteration of codon usage  
11 such that it is maximised for those codons most  
12 frequently used in chickens. For expression in  
13 transgenic chickens the codons of constructs are  
14 optimised for most frequent codon usage in chickens.  
15 However, it can be seen that the optimisation could be  
16 for the most frequent codon usage of any avian species.

17

18 **EXAMPLE**

19 **Chickenising the human IgG Fc DNA sequence**

20 For expression in transgenic chickens the codons of the  
21 chimaeric and humanised minibody versions of R24 are  
22 optimised for most frequent codon usage in chickens  
23 (*Gallus gallus*). A table detailing frequency of codon  
24 usage was downloaded from <http://www.kazusa.or.jp> and is  
25 reproduced in Figure 3.

26

27 For an example of how chickenisation is carried out, it  
28 can be seen that the amino acid Valine is encoded by 4  
29 different codons, GTG, GTA, GTT and GTC with GTG used  
30 most frequently in chickens (46% GTG, 11% GTA, 19% GTT  
31 and 23% GTC\*). To chickenise the human IgG Fc DNA, all  
32 valine codons were converted to GTG. Lysine is encoded  
33 by two different codons, AAG and AAA, with AAG used most

1 frequently in chickens (58% vs 42%). All AAA codons in  
2 the sequence were converted to AAG. Not all codons  
3 required alteration. For example, the two codons for  
4 aspartic acid, GAT and GAC are used with almost equally  
5 (48% vs 52%) and are not changed during the  
6 chickenisation.

7

8 Sequence ID 1 shows the codons for the original human IgG  
9 Fc DNA sequence. Sequence ID 2 shows the chickenised  
10 version of this. Sequence ID 3 shows an alignment of the  
11 nucleotide sequences, a dot indicates a sequence match  
12 and the missing dots show where the codons have been  
13 altered. Sequence ID 4 is an alignment of the amino acid  
14 sequences which show that despite the alterations to  
15 various codons the amino acids are still 100% identical.  
16 Sequence ID 5 and 6 show the chickenised R24 scFv  
17 sequence and complete chickenised R24 minibody  
18 respectively.

19

20 \* figures as given in Figure 3 = 99%

21

## 22 Inserting the Construct into an avian Cell

23

24 There are a number of possible methods that can be used  
25 for transfection of an avian cell with the construct.  
26 Transfection can either be transient or stable.

27

28 In transient transfection, supercoiled plasmid containing  
29 the gene of interest is introduced into the nucleus of  
30 the target cells at high copy number for short periods of  
31 time (usually 24-96 hrs). During transient transfections  
32 the DNA does not integrate into the cellular chromosomes.

33

1 For stable transfection either linear or plasmid DNA can  
2 be introduced into the target cells and will either  
3 integrate into the chromosomes or be maintained as a  
4 stable episome. Linear DNA is optimal for stable  
5 integration but is taken up less efficiently than  
6 supercoiled plasmid. Cells in which the DNA has  
7 integrated or is maintained as a stable episome can be  
8 distinguished by selectable markers. For transfections  
9 with pCIneo, the plasmid carries the neomycin  
10 phosphotransferase gene which confers resistance to  
11 aminoglycosides such as G418. Culturing cells in the  
12 presence of G418 selects for those that carry the  
13 integrated or episomal DNA.

14  
15 A variety of methods are available for the introduction  
16 of DNA into mammalian cells and these include calcium  
17 phosphate coprecipitation (Graham, R.L. and van der Erb,  
18 AJA (1973) Virology 52, 456.) and electroporation  
19 (Andreason, G.L. and Evans, G.A. (1988) BioTechniques 6,  
20 650; Shigikawa, K. and Dover, W.J., (1988) BioTechniques  
21 6, 742) but these have largely been superseded by  
22 cationic liposome-mediated transfection (Felgner, J. et  
23 al (1993) J Tiss Cult Metho. 15, 63). Other compounds  
24 known to mediate transfection of mammalian cells include  
25 lipopolyamines (Remy, J-S, Sirlin, C., Vierling, P and  
26 Behr, J-P. (1994) Bioconjugate Chem. 5, 647) and  
27 dendrimers (Haensler, J. and Szoka, FC (Jr) (1993)  
28 Bioconjugate Chem 4, 372.).

29

30 Cationic liposomes, lipopolyamines and dendrimers coat the  
31 DNA to be transfected and mediate its passage through the  
32 cell membrane. A variety of factors influence the  
33 efficiency of transfection and these include cell type,

1 media type and presence of serum and antibiotics, amount  
2 and quality of plasmid DNA and cytotoxicity of  
3 transfection reagent. All of these usually have to be  
4 optimised for each cell type and plasmid construct.

5

6 Alternatively, zygotic injection of the construct could  
7 also be used to incorporate the construct.

8

9 Alternatively the construct may be cloned into a viral  
10 vector such as a lentivirus vector and such vectors are  
11 commercially available. Lentiviruses as vectors have been  
12 developed from slow retroviruses, such as equine  
13 infectious anaemia virus (EIAV), feline immunodeficiency  
14 virus (FIV) or Human Immunodeficiency Virus (HIV). The  
15 significant advantage using a lentiviral vector is that  
16 the virus will infect cells that are not dividing, which  
17 is appropriate to certain cell types of the present  
18 invention.

19

20 If the construct contains a promoter such as an egg white  
21 protein signal peptide, transgenic avians can then be  
22 produced which lay eggs with the antibody of interest in  
23 the egg white.

24

25 The chickenised construct may also be designed for  
26 insertion into a gene contained within a plasmid, for  
27 example it may be designed for insertion into a lysozyme  
28 gene contained within a plasmid. The ATG site on the  
29 lysozyme gene in the plasmid is destroyed by creating a  
30 SalI site so that the lysozyme protein is not expressed.  
31 The chickenised construct, which has its own ATG can then  
32 be cloned into the SalI site.

33

1 Various modifications may be made to the invention herein  
2 described, without departing from the scope thereof. For  
3 example, any appropriate immunoglobulin sequence may be  
4 used and any appropriate avian species may be used in  
5 place of chickens with the codon bias changing  
6 appropriately.

1 CLAIMS

- 2  
3
- 4 1. A DNA construct which when transfected into an avian  
5 cell will allow the production of an antibody  
6 molecule or functional fragment of said molecule,  
7 and which comprises at least one sequence comprising  
8 the variable domain of an immunoglobulin heavy chain  
9 and at least one sequence comprising the variable  
10 domain of an immunoglobulin light chain, and wherein  
11 the DNA construct is based on a non-avian sequence  
12 and one or more of the codons in the DNA construct  
13 have been altered such that for the amino acid being  
14 encoded, the codon used is that which most  
15 frequently appears in avians.  
16
- 17 2. A DNA construct as claimed in Claim 1, wherein the  
18 construct also contains an avian signal peptide  
19 sequence.  
20
- 21 3. A construct as claimed in the previous Claims,  
22 wherein the construct is cloned into a viral vector.  
23
- 24 4. A construct as described in Claim 3, wherein the  
25 viral vector is a lentivirus vector.  
26
- 27 5. A DNA construct as claimed in Claims 2 to 4, wherein  
28 the avian signal peptide sequence is a signal  
29 peptide sequence from an egg white protein.  
30
- 31 6. A DNA construct as described in Claim 5, wherein the  
32 egg white protein is chosen from the list lysozyme,  
33 ovalbumin, ovatransferrin or ovomucoid.  
34

- 1 7. A DNA construct as claimed in any of the previous  
2 Claims, wherein the construct also includes  
3 immunoglobulin constant regions for dimerisation and  
4 recruitment of effector functions.  
5
- 6 8. A DNA construct as claimed in Claim 7, wherein the  
7 immunoglobulin constant regions are CH2 and CH3.  
8
- 9 9. A DNA construct as claimed in Claims 7 or 8, wherein  
10 the immunoglobulin constant regions are human  
11 constant regions.  
12
- 13 10. A DNA construct as described in any of the previous  
14 Claims, wherein the construct may be transfected  
15 into an avian cell using electroporation.  
16
- 17 11. A DNA construct as claimed in Claims 1 to 9, wherein  
18 the construct may be transfected into an avian cell  
19 using lipofection.  
20
- 21 12. A DNA construct as claimed in Claim 1 to 9, wherein  
22 the construct may be directly injected into the  
23 nucleus of an avian.  
24
- 25 13. A DNA construct as claimed in Claim 12, wherein the  
26 construct may be directly injected into the germinal  
27 disc of an oocyte.  
28
- 29 14. A DNA construct as described in any of the previous  
30 Claims, wherein codon usage in the construct is  
31 maximised for those codons most frequently appearing  
32 in avians.  
33

- 1 15. An avian cell containing the construct described in  
2 Claims 1 to 15, which expresses an immunoglobulin  
3 molecule or functional fragment of said molecule.  
4
- 5 16. An avian cell as described in Claim 16, wherein the  
6 expressed immunoglobulin molecule or functional  
7 fragment thereof shows an avian glycosylation  
8 pattern.  
9
- 10 17. An avian cell as described in Claims 16 or 17,  
11 wherein the immunoglobulin or fragment thereof is  
12 expressed at a higher expression level than a  
13 standard human construct or humanised construct.  
14
- 15 18. A method for producing avian cells capable of  
16 expressing an immunoglobulin molecule or functional  
17 fragment of said molecule, comprising transfecting  
18 an avian cell with the DNA construct as described in  
19 Claims 1 to 15.  
20
- 21 19. Preferably the avian cell is selected from a list of  
22 chicken cell, duck cell, turkey cell, quail cell or  
23 ostrich cell.  
24
- 25 20. An immunoglobulin or functional fragment thereof  
26 which is produced using the method described in  
27 Claims 19 and 20.  
28
- 29 21. A transgenic avian which expresses the construct  
30 described in Claims 1 to 15.  
31



- 1 22. A transgenic avian as described in Claim 22, wherein  
2 the molecule coded for by the construct is expressed  
3 in the egg of the transgenic avian..  
4
- 5 23. A transgenic avian as claimed in Claim 23, wherein  
6 the construct is expressed in the egg white.  
7
- 8 24. A transgenic avian as described in Claim 23, wherein  
9 the construct is expressed in the egg yolk.  
10
- 11 25. A transgenic avian as described in Claims 22 to 25,  
12 wherein the expressed immunoglobulin shows an avian  
13 glycosylation pattern.

1/5

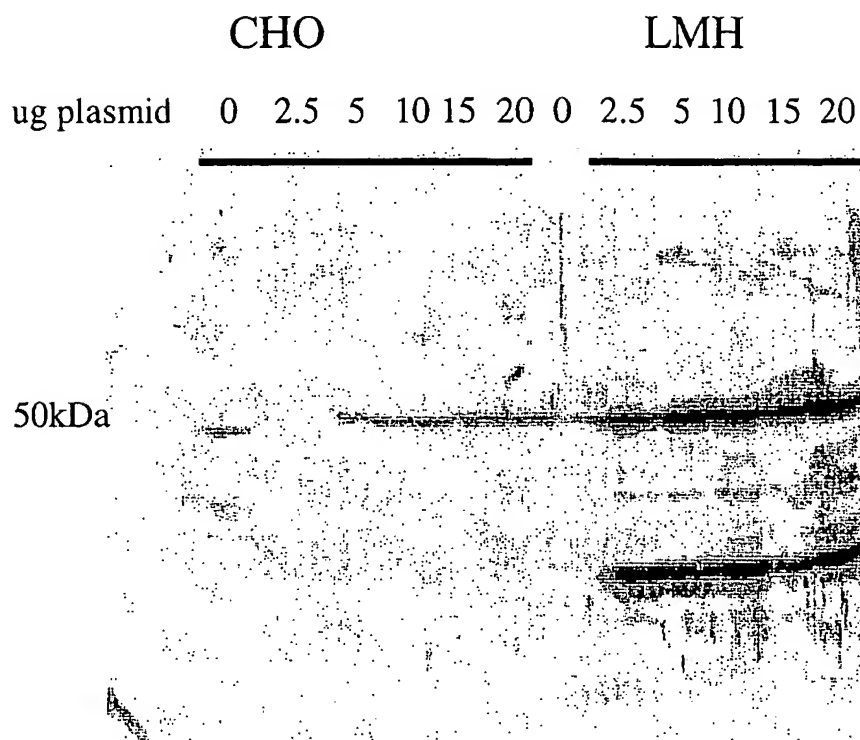


Figure 1: Equal numbers of cells were transfected with increasing amounts of chickenised R24 in pCIneo. Expression was detected by Western blotting using anti-human IgG Fc

2/5

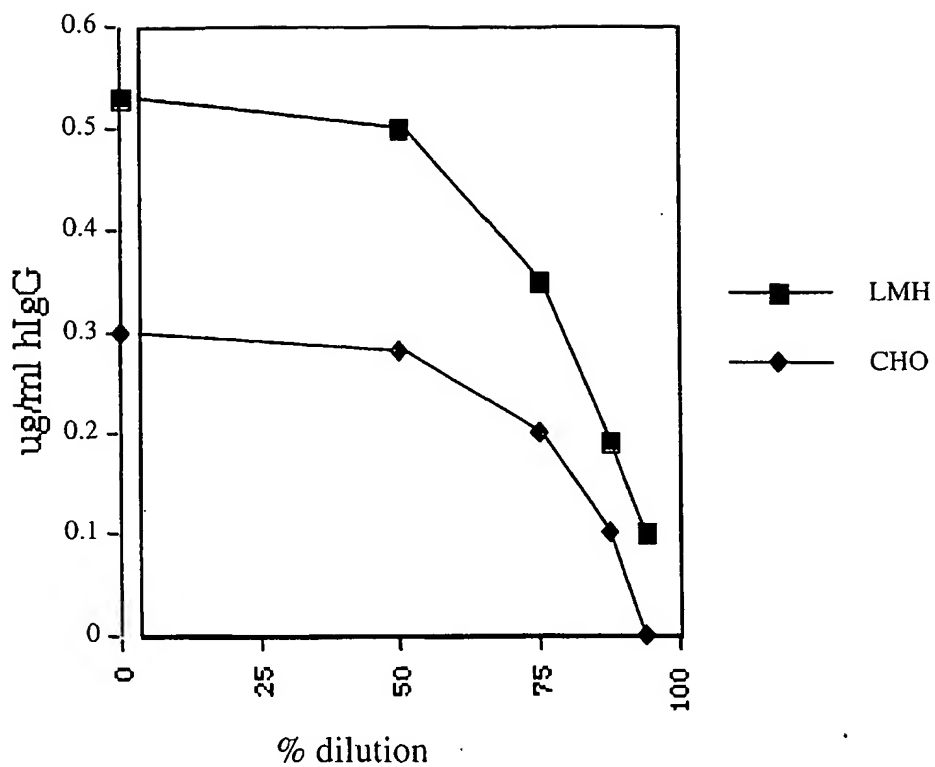


Figure 2: Concentrations of human IgG in culture medium from cells transiently transfected with 4ug p7.2  
Concentration of chimaeric R24 minibody was determined by human IgG1 ELISA.  
For % dilution, 0 = undiluted medium

3/5

Figure 3: Gallus gallus [gbvrt]: 1867 CDS's (902768 codons)

A m A c i d	C o d o n	N u m b e r	/1000	F r a c t i o n
Gly	GGG	15151.00	16.78	0.25
Gly	GGA	15334.00	16.99	0.26
Gly	GGT	10067.00	11.15	0.17
Gly	GGC	19197.00	21.26	0.32
Glu	GAG	39237.00	43.46	0.59
Glu	GAA	27671.00	30.65	0.41
Asp	GAT	21825.00	24.18	0.48
Asp	GAC	23834.00	26.40	0.52
Val	GTG	25842.00	28.63	0.46
Val	GTA	6430.00	7.12	0.11
Val	GTT	10831.00	12.00	0.19
Val	GTC	13180.00	14.60	0.23
Ala	GCG	8155.00	9.03	0.13
Ala	GCA	15732.00	17.43	0.24
Ala	GCT	18019.00	19.96	0.28
Ala	GCC	22576.00	25.01	0.35
Arg	AGG	10422.00	11.54	0.21
Arg	AGA	10268.00	11.37	0.21
Ser	AGT	9108.00	10.09	0.13
Ser	AGC	18604.00	20.61	0.27
Lys	AAG	32939.00	36.49	0.58
Lys	AAA	23618.00	26.16	0.42
Asn	AAT	14361.00	15.91	0.40
Asn	AAC	21629.00	23.96	0.60

4/5

AmAcid	Codon	Number	/1000	Fraction
Met	ATG	21093.00	23.36	1.00
Ile	ATA	7094.00	7.86	0.17
Ile	ATT	14280.00	15.82	0.33
Ile	ATC	21332.00	23.63	0.50
Thr	ACG	7340.00	8.13	0.15
Thr	ACA	14212.00	15.74	0.28
Thr	ACT	11545.00	12.79	0.23
Thr	ACC	16795.00	18.60	0.34
Trp	TGG	10535.00	11.67	1.00
End	TGA	935.00	1.04	0.43
Cys	TGT	7336.00	8.13	0.37
Cys	TGC	12519.00	13.87	0.63
End	TAG	482.00	0.53	0.22
End	TAA	737.00	0.82	0.34
Tyr	TAT	10021	11.1	0.37
Tyr	TAC	17114	18.96	0.63
Leu	TTG	10357.00	11.47	0.13
Leu	TTA	5406.00	5.99	0.07
Phe	TTT	13896.00	15.39	0.42
Phe	TTC	18856.00	20.89	0.58
Ser	TCG	4956.00	5.49	0.07
Ser	TCA	9525.00	10.55	0.14
Ser	TCT	11639.00	12.89	0.17
Ser	TCC	15048.00	16.67	0.22

5/5

A mAcid	Codon	Number	/1000	Fraction
Arg	CGG	8815.00	9.76	0.18
Arg	CGA	4502.00	4.99	0.09
Arg	CGT	4815.00	5.33	0.10
Arg	CGC	10528.00	11.66	0.21
Gln	CAG	29180.00	32.32	0.73
Gln	CAA	10558.00	11.70	0.27
His	CAT	7845.00	8.69	0.37
His	CAC	13525.00	14.98	0.63
Leu	CTG	34916.00	38.68	0.43
Leu	CTA	4886.00	5.41	0.06
Leu	CTT	9750.00	10.8	0.12
Leu	CTC	15185.00	16.82	0.19
Pro	CCG	7617.00	8.44	0.15
Pro	CCA	13515.00	14.97	0.26
Pro	CCT	12986.00	14.38	0.25
Pro	CCC	17062.00	18.90	0.33

1  
2  
3 **Sequence ID 1. The sequence of human IgG Fc used**  
4 **for construction of chimaeric and humanised**  
5 **minibodies.**  
6  
7 AC CTT GCA GGA TCC GCA AGA CCC AAA TCT  
8 TGT  
9  
10 GAC AAA ACT CAC ACA TGC CCA CCG TGC  
11 CCA GCA  
12  
13 CCT GAA CTC CTG GGG GGA CCG TCA GTC  
14 TTC CTC  
15  
16 TTC CCC CCA AAA CCC AAG GAC ACC CTC  
17 ATG ATC  
18  
19 TCC CGG ACC CCT GAG GTC ACA TGC GTG  
20 GTG GTG  
21  
22 GAC GTG AGC CAC GAA GAC CCT GAG GTC  
23 AAG TTC  
24  
25 AAC TGG TAC GTG GAC GGC GTG GAG GTG  
26 CAT AAT  
27  
28 GCC AAG ACA AAG CCG CGG GAG GAG CAG  
29 TAC AAC  
30  
31 AGC ACG TAC CGG GTG GTC AGC GTC CTC  
32 ACC GTC  
33

1 CTG CAC CAG GAC TGG CTG AAT GGC AAG  
2 GAG TAC  
3  
4 AAG TGC AAG GTC TCC AAC AAA GCC CTC  
5 CCA GCC  
6  
7 CCC ATC GAG AAA ACC ATC TCC AAA GCC  
8 AAA GGG  
9  
10 CAG CCC CGA GAA CCA CAG GTG TAC ACC  
11 CTG CCC  
12  
13 CCA TCC CGG GAG GAG ATG ACC AAG AAC  
14 CAG GTC  
15  
16 AGC CTG ACC TGC CTG GTC AAA GGC TTC  
17 TAT CCC  
18  
19 AGC GAC ATC GCC GTG GAG TGG GAG AGC  
20 AAT GGG  
21  
22 CAG CCG GAG AAC AAC TAC AAG ACC ACG  
23 CCT CCC  
24  
25 GTG CTG GAC TCC GAC GGC TCC TTC TTC  
26 CTC TAT  
27  
28 AGC AAG CTC ACC GTG GAC AAG AGC AGG  
29 TGG CAG  
30  
31 CAG GGG AAC GTC TTC TCA TGC TCC GTG  
32 ATG CAT  
33



1 GAG GCT CTG CAC AAC CAC TAC ACG CAG  
2 AAG AGC  
3  
4 CTC TCC CTG TCC CCG GGT AAA TGA TAA  
5 GTC GAC  
6  
7 ACG TGA TC  
8

1 **Sequence ID 2. The chickenised human IgG Fc DNA**  
2 **sequence.**

3 Codon alterations are in red.

4

5

6 AC CTT GCA GGA TCC GCC AGA CCC AAG TC  
7 TGC

8

9 GAC AAG ACC CAC ACA TGC CCA CCC TGC  
10 CCA GCC

11

12 CCC GAG CTG CTG GGG GGA CCC TCC GTG  
13 TTC CTG

14

15 TTC CCC CCA AAG CCC AAG GAC ACC CTG  
16 ATG ATC

17

18 TCC CGC ACC CCC GAG GTG ACA TGC GTG  
19 GTG GTG

20

21 GAC GTG AGC CAC GAG GAC CCC GAG GTG  
22 AAG TTC

23

24 AAC TGG TAC GTG GAC GGC GTG GAG GTG  
25 CAC AAC

26

27 GCC AAG ACA AAG CCC CGC GAG GAG CAG  
28 TAC AAC

29

30 AGC ACC TAC CGC GTG GTG AGC GTG CTG  
31 ACC GTG

32

33 CTG CAC CAG GAC TGG CTG AAC GGC AAG  
34 GAG TAC

1  
2 AAG TGC AAG GTG TCC AAC AAG GCC CTG  
3 CCA GCC  
4  
5 CCC ATC GAG AAG ACC ATC TCC AAG GCC  
6 AAG GGG  
7  
8 CAG CCC CGC GAG CCA CAG GTG TAC ACC  
9 CTG CCC  
10  
11 CCA TCC CGC GAG GAG ATG ACC AAG AAC  
12 CAG GTG  
13  
14 AGC CTG ACC TGC CTG GTG AAG GGC TTC  
15 TAC CCC  
16  
17 AGC GAC ATC GCC GTG GAG TGG GAG AGC  
18 AAC GGG  
19  
20 CAG CCC GAG AAC AAC TAC AAG ACC ACC  
21 CCC CCC  
22  
23 GTG CTG GAC TCC GAC GGC TCC TTC TTC  
24 CTG TAC  
25  
26 AGC AAG CTG ACC GTG GAC AAG AGC AGG  
27 TGG CAG  
28  
29 CAG GGG AAC GTG TTC TCC TGC TCC GTG  
30 ATG CAC  
31  
32 GAG GCC CTG CAC AAC CAC TAC ACC CAG  
33 AAG AGC

1  
2 CTC TCC CTG TCC CCC GGC AAG TGA TAA  
3 GTG GAC  
4  
5 ACC TGA TC  
6

1  
2 **Sequence ID 3. Nucleotide alignment of the**  
3 **original (upper) and chickenised (lower) human**  
4 **IgG Fc.**  
5  
6           10       20       30       40       50       60  
7 70  
8 |  
9 |  
10 ACCTTGCAAGGATCCGCAAGACCCAAATCTTGTGACAAAACACACATGCCACCGTGCCAGCACCTG  
11 AACTCCTG  
12 .....  
13 ..  
14 ACCTTGCAAGGATCCGCAAGACCCAAATCTTGTGACAAAACACACATGCCACCGTGCCAGCCCCG  
15 AGCTCCTG  
16 |  
17 |  
18           10       20       30       40       50       60  
19 70  
20  
21  
22  
23       80       90       100       110       120       130       140  
24 150  
25 |  
26 |  
27 GGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAGGACACCCCTCATGATCTCCCGGACCCCTGAG  
28 GTCACATG  
29 .....  
30 .....  
31 GGGGGACCCCTCCGTGTTCTCTTCCCCCAAAAGCCCAAGGACACCCCTCATGATCTCCCGGACCCCGAG  
32 GTGACATG  
33 |  
34 |  
35       80       90       100       110       120       130       140  
36 150  
37  
38  
39  
40       160       170       180       190       200       210       220  
41 230  
42 |  
43 |  
44 CGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT  
45 GCATAATG  
46 .....  
47 .....  
48 CGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGT  
49 GCACAACG  
50 |  
51 |  
52       160       170       180       190       200       210       220  
53 230  
54  
55  
56

```
1      240      250      260      270      280      290
2      300
3      |
4      CCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACGTACCGGGTCCTCAGCGTCCTCACCCTCCTGC
5      ACCAGCAC
6      .....
7      .....
8      CCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGC
9      ACCAGCAC
10
11      240      250      260      270      280      290
12      300
13
14
15
16      310      320      330      340      350      360      370
17      380
18      |
19      |
20      TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
21      ATCTCCAA
22      .....
23      .....
24      TGGCTGAACGSCAAGGAGTACAAGTGCAAGGTGTCCAACAAAGCCCTGCCAGCCCCCATCGAGAAGACC
25      ATCTCCAA
26      |
27      |
28      310      320      330      340      350      360      370
29      380
30
31
32
33      390      400      410      420      430      440      450
34      460
35      |
36      |
37      AGCCAAAGGGGAGCCCCGAGAACACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAA
38      CCAGGTCA
39      .....
40      .....
41      GGCCAAGGGGAGCCCCGAGCCACAGGTGTACACCCTGCCCCATCCCGCGAGGAGATGACCAAGAA
42      CCAGGTGA
43      |
44      |
45      390      400      410      420      430      440      450
46      460
47
48
49
50      470      480      490      500      510      520
51      530
52      |
53      GCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
54      CGGAGAAC
55      .....
56      .....
57      GCCTGACCTGCCTGGTGAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGGCAGC
58      CCGAGAAC
59      |
```

1 470 480 490 500 510 520  
2 530  
3  
4  
5  
6 540 550 560 570 580 590 600  
7 610  
8 | | | | | |  
9 |  
10 AACTACAAGACCACGCCCTCCCGTGGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTG  
11 GACAAGAG  
12 .....  
13 .....  
14 AACTACAAGACCCCCCGTGGCTGGACTCCGACGGCTCCTTCTTCCTGTACAGCAAGCTGACCGTG  
15 GACAAGAG  
16 | | | | | |  
17 |  
18 540 550 560 570 580 590 600  
19 610  
20  
21  
22  
23 620 630 640 650 660 670 680  
24 690  
25 | | | | | |  
26 |  
27 CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCA  
28 GAACAGCC  
29 .....  
30 .....  
31 CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCA  
32 GAAGAGCC  
33 | | | | | |  
34 |  
35 620 630 640 650 660 670 680  
36 690  
37  
38  
39  
40 700 710 720 730  
41 | | | |  
42 TCTCCCTGTCCCCGGTAAATGATAAGTCGACACGTGATC  
43 .....  
44 TCTCCCTGTCCCCGGCAAGTGATAAGTGGACACCTGATC  
45 | | | |  
46 700 710 720 730  
47  
48  
49  
50

1  
2 **Sequence ID4. Amino acid alignment of original**  
3 **(upper) and chickenised (lower) human IgG Fc.**  
4  
5           10           20           30           40           50           60  
6 70           |           |           |           |           |           |  
7  
8 |  
9 LAGSARPKSCDKTHTCPFCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV  
10 DGVEVHNA  
11 .....  
12 .....  
13 LAGSARPKSCDKTHTCPFCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV  
14 DGVEVHNA  
15 |           |           |           |           |           |  
16 |  
17           10           20           30           40           50           60  
18 70           |           |           |           |           |           |  
19  
20  
21  
22       80       90       100       110       120       130       140  
23 150  
24 |           |           |           |           |           |  
25 |  
26 KTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE  
27 EMTKNQVS  
28 .....  
29 .....  
30 KTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE  
31 EMTKNQVS  
32 |           |           |           |           |           |  
33 |  
34       80       90       100       110       120       130       140  
35 150  
36  
37  
38  
39       160       170       180       190       200       210       220  
40 230  
41 |           |           |           |           |           |  
42 |  
43 LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH  
44 NHYTQKSL  
45 .....  
46 .....  
47 LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH  
48 NHYTQKSL  
49 |           |           |           |           |           |  
50 |  
51       160       170       180       190       200       210       220  
52 230  
53  
54  
55  
56       240  
57 |  
58 SLSPGK...VDT



1 .....  
2 SLSPGK..VDT  
3           !  
4           240  
5  
6  
7

1

2

3 Sequence ID5. Chickenised R24 sequence. Altered  
4 codons are shown in red. The underlined  
5 nucleotides encode the lysozyme leader amino  
6 acids

7

8 GGC CGG GTC GAC ATG AGG TCT TTG CTA  
9 ATC TTG

10

11 GTG CTT TGC TTC CTG CCC CTG GCT GCT  
12 CTG GGG

13

14 GAT GTG CAG CTG GTG GAG TCC GGG GGA  
15 GGC CTG

16

17 GTG CAG CCC GGA GGG TCC CGC AAG CTC  
18 TCC TGC

19

20 GCC GCC TCC GGA TTC ACC TTC AGC AAC  
21 TTC GGA

22

23 ATG CAC TGG GTG CGC CAG GCC CCC GAG  
24 AAG GGG

25

26 CTG GAG TGG GTG GGA TAC ATC AGC AGC  
27 GGC GGC

28

29 AGC TCC ATC AAC TAC GCC GAC ACC GTG  
30 AAG GGC

31

32 CGC TTC ACC ATC TCC AGA GAC AAC CCC  
33 AAG AAC

34

1 ACC CTG TTC CTG CAG ATG ACC AGC CTG  
2 AGG TCC  
3  
4 GAG GAC ACA GCC ATC TAC TAC TGC ACC  
5 AGA GGG  
6  
7 GGA ACC GGG ACC AGA TCC CTG TAC TAC  
8 TTC GAC  
9  
10 TAC TGG GGC CAG GGC GCC ACA CTG ATC  
11 GTG TCC  
12  
13 TCC GGG GGA GGC GGC TCC GGG GGA GGC  
14 GGC TCC  
15  
16 GGG GGA GGC GGC TCC GAT ATC CAG ATG  
17 ACA CAG  
18  
19 ATC ACA TCC TCC CTG TCT GTG TCT CTG  
20 GGA GAC  
21  
22 AGA GTG ATC ATC AGC TGC AGG GCT AGC  
23 CAG GAC  
24  
25 ATC GGC AAT TTT CTG AAC TGG TAC CAG  
26 CAG GAA  
27  
28 CCA GAT GGA TCT CTG AAG CTG CTG ATC  
29 TAC TAC  
30  
31 ACA TCT AGA CTG CAG TCC GGA GTG CCA  
32 TCC AGG  
33

1 TTC AGC GGC TGG GGG TCT GGA ACA GAT  
2 TAC TCT  
3  
4 CTG ACC ATT AGC AAC CTG GAG GAA GAG  
5 GAT ATC  
6  
7 GCC ACC TTC TTC TGC CAG CAG GGC AAG  
8 ACA CTG  
9  
10 CCC TAC ACC TTC GGA GGG GGG ACC AAG  
11 CTG GAG  
12  
13 ATC AAG CGC GGA TCC GCC GCC G

1 Sequence ID6. The complete chickenised nucleotide  
2 sequence of the R24 chimaeric minibody.  
3  
4 GGC CGG GTC GAC ATG AGG TCT TTG CTA  
5 ATC TTG  
6  
7 GTG CTT TGC TTC CTG CCC CTG GCT GCT  
8 CTG GGG  
9  
10 GAT GTG CAG CTG GTG GAG TCC GGG GGA  
11 GGC CTG  
12  
13 GTG CAG CCC GGA GGG TCC CGC AAG CTC  
14 TCC TGC  
15  
16 GCC GCC TCC GGA TTC ACC TTC AGC AAC  
17 TTC GGA  
18  
19 ATG CAC TGG GTG CGC CAG GCC CCC GAG  
20 AAG GGG  
21  
22 CTG GAG TGG GTG GGA TAC ATC AGC AGC  
23 GGC GGC  
24  
25 AGC TCC ATC AAC TAC GCC GAC ACC GTG  
26 AAG GGC  
27  
28 CGC TTC ACC ATC TCC AGA GAC AAC CCC  
29 AAG AAC  
30  
31 ACC CTG TTC CTG CAG ATG ACC AGC CTG  
32 AGG TCC  
33

1 GAG GAC ACA GCC ATC TAC TAC TGC ACC  
2 AGA GGG  
3  
4 GGA ACC GGG ACC AGA TCC CTG TAC TAC  
5 TTC GAC  
6  
7 TAC TGG GGC CAG GGC GCC ACA CTG ATC  
8 GTG TCC  
9  
10 TCC GGG GGA GGC GGC TCC GGG GGA GGC  
11 GGC TCC  
12  
13 GGG GGA GGC GGC TCC GAT ATC CAG ATG  
14 ACA CAG  
15  
16 ATC ACA TCC TCC CTG TCT GTG TCT CTG  
17 GGA GAC  
18  
19 AGA GTG ATC ATC AGC TGC AGG GCT AGC  
20 CAG GAC  
21  
22 ATC GGC AAT TTT CTG AAC TGG TAC CAG  
23 CAG GAA  
24  
25 CCA GAT GGA TCT CTG AAG CTG CTG ATC  
26 TAC TAC  
27  
28 ACA TCT AGA CTG CAG TCC GGA GTG CCA  
29 TCC AGG  
30  
31 TTC AGC GGC TGG GGC TCT GGA ACA GAT  
32 TAC TCT  
33

1 CTG ACC ATT AGC AAC CTG GAG GAA GAG  
2 GAT ATC  
3  
4 GCC ACC TTC TTC TGC CAG CAG GGC AAG  
5 ACA CTG  
6  
7 CCC TAC ACC TTC GGA GGG GGG ACC AAG  
8 CTG GAG  
9  
10 ATC AAG CGC GGA TCC GCC AGA CCC AAG  
11 TCC TGC  
12  
13 GAC AAG ACC CAC ACA TGC CCA CCC TGC  
14 CCA GCC  
15  
16 CCC GAG CTG CTG GGG GGA CCC TCC GTG  
17 TTC CTG  
18  
19 TTC CCC CCA AAG CCC AAG GAC ACC CTG  
20 ATG ATC  
21  
22 TCC CGC ACC CCC GAG GTG ACA TGC GTG  
23 GTG GTG  
24  
25 GAC GTG AGC CAC GAG GAC CCC GAG GTG  
26 AAG TTC  
27  
28 AAC TGG TAC GTG GAC GGC GTG GAG GTG  
29 CAC AAC  
30  
31 GCC AAG ACA AAG CCC CGC GAG GAG CAG  
32 TAC AAC  
33

1 AGC ACC TAC CGC GTG GTG AGC GTG CTG  
2 ACC GTG  
3  
4 CTG CAC CAG GAC TGG CTG AAC GGC AAG  
5 GAG TAC  
6  
7 AAG TGC AAG GTG TCC AAC AAG GCC CTG  
8 CCA GCC  
9  
10 CCC ATC GAG AAG ACC ATC TCC AAG GCC  
11 AAG GGG  
12  
13 CAG CCC CGC GAG CCA CAG GTG TAC ACC  
14 CTG CCC  
15  
16 CCA TCC CGC GAG GAG ATG ACC AAG AAC  
17 CAG GTG  
18  
19 AGC CTG ACC TGC CTG GTG AAG GGC TTC  
20 TAC CCC  
21  
22 AGC GAC ATC GCC GTG GAG TGG GAG AGC  
23 AAC GGG  
24  
25 CAG CCC GAG AAC AAC TAC AAG ACC ACC  
26 CCC CCC  
27  
28 GTG CTG GAC TCC GAC GGC TCC TTC TTC  
29 CTG TAC  
30  
31 AGC AAG CTG ACC GTG GAC AAG AGC AGG  
32 TGG CAG  
33



1 CAG GGG AAC GTG TTC TCC TGC TCC GTG  
2 ATG CAC  
3  
4 GAG GCC CTG CAC AAC CAC TAC ACC CAG  
5 AAG AGC  
6  
7 CTC TCC CTG TCC CCC GGC AAG TGA TAA  
8 GTC GAC  
9  
10 ACC TGA TC  
11